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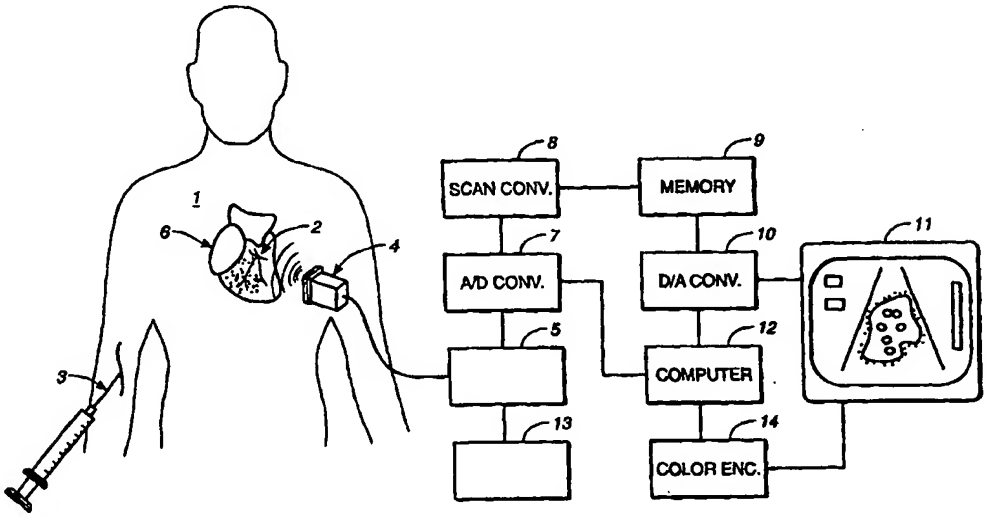

(57) Abstract

A method is provided for delivering pharmacological agents to a region within the subject (1) by introducing microcapsule (2) containing the agent into the bloodstream, where the microcapsule (2) has specific, and precise acoustical characteristics. The microcapsule (2) is introduced into the bloodstream of the subject (1), its location is monitored to determine its presence at the region, then ultrasonic energy from ultrasonic probe (4) is directed at the location at a frequency waveform or intensity sufficient to induce the release of the pharmacological agent at the region to achieve a pharmaceutical effect.

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METHOD FOR ULTRASOUND TRIGGERED DRUG DELIVERY

This is a continuation-in-part of Serial No. 09/020,046, filed February 6, 1998.

Background of the Invention

The current invention relates to the fields of ultrasonic, diagnostic and therapeutic delivery of agents to a localized region within a subject.

Ultrasound imaging has a wide application in the field of medical practice. Ultrasonic diagnostics refers to the imaging of the region of the human or animal patient using an ultrasound transducer to generate and receive ultrasonic waves. Typically, the transducer is placed on the patient's body over a region to be imaged and high frequency sound waves are generated by the transducer and directed at the region. The transducer receives reflected ultrasonic waves from the region and converts the received waves into electrical signals from which an image is generated. Due to the extremely high acoustic reflectivity of gases, contrast agents comprised of gas bubbles with and without encapsulating shells may be used to improve the quality of ultrasound images by highlighting the blood pool and the vascular perfusion of organs within the body. Exemplary contrast agents include, for example, stabilized microbubbles, sonicated albumin, gas-filled microspheres, gas-filled liposomes and gas forming emulsions.

The use of an ultrasound contrast agent which may also serve as a drug carrier has been described for gas-filled liposomes in US Patent 5,580,575. A quantity of liposomes containing drug is administered into the circulatory system of a patient and monitored using ultrasonic energy at diagnostic levels, until the presence of the liposomes are detected in the region of interest. Ultrasonic energy is then applied to the region that is sufficient to rupture the liposomes to release drugs locally for therapeutic purposes. The ultrasonic energy is described in US Patent 5,558,082 to be applied by a transducer that simultaneously applies diagnostic and therapeutic ultrasonic waves from therapeutic transducer elements located centrally to the diagnostic transducer elements.

The use of gas-filled microcapsules to control the delivery of drugs to a region of the body has also been described in US Patent 5,190,766 in which the acoustic resonance frequency of the drug carrier is measured in the region in which the drug is to be released and then the region is irradiated with the appropriate sound wave to control the release of drug. Separate ultrasound transducers are described for the imaging and triggering of drug release in the target region.

The combination of imaging and therapeutic means into an ultrasound system involves distinct acoustical functions. Therapeutic ultrasound is typically carried out at different frequencies from diagnostic ultrasound, since it is desirable to perform therapeutic ultrasound at lower frequencies in order to achieve low attenuation and deeper tissue penetration, whereas higher frequencies are employed in diagnostic ultrasound to obtain better resolution. Due to the low mechanical properties of the liposome membrane, the ultrasonic frequencies utilized to rupture liposomes are in the range of 1 - 2 MHZ, similar to free gas bubbles in the range of 2-8 microns diameter. The ultrasonic intensity and wave form which might be effective for rupturing microparticles or microcapsules having stiff shells with mechanical properties greater than liposomal membranes presents other considerations. Microparticles or microcapsules fabricated from polymers or other viscoelastic materials have additional complexity due to time variant acoustical characteristics due to viscous damping. For example, in U.S. Patent 5,190,766, hollow microcapsules made of albumin containing a bioactive agent were irradiated with ultrasonic waves of 1.2 to 2 MHZ and an intensity of 200 mW/cm^2 to rupture and release the bioactive agent in the circulation of the body. However, the acoustic characteristics of the bioactive agent or the microcapsule had to be measured in the region in which the agent was to be released in order to calculate the resonance frequency. This requires situating the microcapsules in the desired release region in the body in order to take measurements and to calculate the resonance frequency required for the input ultrasound wave required to rupture the microcapsules.

Other forms of ultrasound have presented means for focusing of the ultrasonic energy to provide a localized therapeutic tissue effect. Designs and methods for the use of ultrasound to destroy stones, provide hyperthermia, and enhance tumor

treatment agents have described the use of ultrasound focusing to localize the treatment tissues. Approaches for therapeutic ultrasound focusing include the use of multiple transducers as described in US Patent 5,005,579 and US Patent 4,441,486, although they may be combined into a central unit as described in US Patent 5,233,994.

Summary of the Invention

The present invention provides a method of delivering therapeutic agents to a localized region within a subject comprising the steps of introducing microcapsules with specific and precise ultrasound drug release characteristics containing an active agent into the blood stream of the subject where said microcapsules comprise a structure, such that their thickness, density and composition allow the specified ultrasonic intensity, frequency or wave form required to rupture the microcapsules at a predetermined threshold condition; monitoring the location of the microcapsules within the subject to determine their presence at the region; and directing at the region ultrasonic energy at a frequency, wave form or intensity sufficient to induce the rupture of the microcapsules and subsequent release of the active agent into the region to achieve the therapeutic effect.

An advantage of the invention is that the microcapsules have specific and predetermined acoustic properties such that the specific ultrasonic energy format required to rupture the microcapsules can be predetermined as a release threshold prior to injection into the subject. In addition, microcapsules can be tailored for specific rupture characteristics to allow use of ultrasound conditions which will not cause rupture except in the desired body region. For example, very fragile drug containing agents would have the undesired release of drugs along the path of the targeting ultrasound beam when deep tissues are targeted. Polymers in particular have robust and tailorable mechanical properties to provide resistance to breakage until appropriate acoustic conditions are reached and allow the fabrication of microcapsules with tailored fragility to ultrasound.

The localization aspect of drug release by ultrasound from circulating microspheres or microcapsules is a significant deviation from the use of ultrasound for

diagnostic imaging. In diagnostic ultrasound imaging, the entire tissue area from the point of transducer contact to many centimeters deep of tissue can be imaged, even though the highest ultrasound intensity is generally nearest the transducer and declines rapidly with depth due to tissue attenuation of approximately 0.3 dB/cm-MHZ. However, in the treatment of a tumor with ultrasound triggered release of cytotoxic compounds, the release is desired to be localized to the tumor, not the overlying tissues with potentially greater ultrasound exposure.

The specific ultrasonic energy may be in the format of a frequency, intensity or wave form. In one embodiment the ultrasonic energy may be produced by a plurality of emitting transducers focused at the region so the additive wave superimposition at the point of convergence of the ultrasonic beams creates an local intensity or condition sufficient to rupture the microcapsules. A separate diagnostic imaging transducer may be used to image the region for treatment. Alternatively a single ultrasound transducer may be used for imaging and triggering the microcapsules by focusing the beam by real time electronic techniques intermittently between rapid diagnostic scans. A seemingly continuous imaging and treatment of the target tissues would result.

The drug containing microcapsules can be imaged with ultrasound under clinically accepted diagnostic power levels for patient safety, below approximately 190 W/cm2 of derated spatial-peak pulse-average intensity or a 1.9 mechanical index. While not required, it is preferred that the microcapsules be rupturable for drug release at threshold power levels below the clinically accepted power levels for diagnosis. Specific matching of ultrasound conditions and microcapsule response to such conditions are important factors in achieving such controlled release conditions. Preferred threshold conditions for rupture are those at power, frequency and wave from sufficient to provide a mechanical index of about 0.2, 0.5, 1.0 or 1.5.

In another embodiment the ultrasonic energy may be generated at the specific resonant frequency of the microcapsules and upon application cause the microcapsules to resonate to the point of structural failure. Alternatively, the microcapsules may be made to resonate at higher and/or lower harmonic ratios of a primary frequency such that the ultrasonic energy may be provided by transducers

which emit the harmonics to rupture the microcapsules within the region.

Typically, it is more difficult to utilize harmonics to rupture microcapsules since gas bubbles are much less responsive to harmonics as compared to the fundamental resonant frequency ("Numerical investigation of nonlinear oscillations of gas bubbles in liquids", Werner Lauterborn, J. Acoustic Society American, Vol. 59, No. 2, Feb. 1976). Harmonically driven oscillation is more difficult with microcapsules with significant mechanical properties or viscoelastic shell characteristics as compared to free gas bubbles.

In another embodiment, the ultrasonic energy is provided by an alteration in the normal diagnostic imaging wave form within the ultrasound pulse or by specific alternate wave form to induce high amplitude resonance which ruptures the microcapsules.

In another embodiment an ultrasonic transducer within the distal section of a cannula which has been introduced into a blood vessel is used to disrupt the microcapsules as they flow past the transducer section, thereby delivering an agent downstream of the cannula. Alternatively, an ultrasound transducer may be implanted within the body or fixated on the body near the target site, to be activated at such times as necessary to treat the site after injection of the microsphere delivery agent.

Brief Description of the Drawings

Fig. 1 is a block diagram of an apparatus used for an embodiment of the present invention.

Fig. 2 is a diagram of a multiple focus transducer for imaging and triggering of drug release from microcapsules.

Fig. 3 is a graph of experimental results indicating the effect of ultrasound energy intensity on a microsphere of this invention.

Fig. 4 is a graph of experimental results indicating the effect of the number of wave periods in the pulsed ultrasound signal on a microsphere of this invention.

Fig. 5 is a graph of experimental results indicating the effect of pulse length on bubble backscattering.

Detailed Description

The microcapsules according to the present invention have an integral outer shell and have specific acoustical characteristics. One or more of the layers of the shell may be comprised of a pharmacological agent or agent-carrying reservoir, or the central core may carry the agent, which may fill the core or be present as a reticulated network within the core. It is preferred, however, that the central core be hollow, i.e., gas-containing, so that the microcapsules may have the dual function of being echogenic to serve as an ultrasound contrast agent within the bloodstream of the subject, and also serving as a drug carrier. For use as ultrasound contrast agents, the cores of the microcapsules contain a physiologically compatible gas such as air. Microcapsules are constructed as described such that the majority will have diameters within the range of about one to ten microns in order to pass through the capillary system of the body and to allow free circulation. The free circulation of the microcapsules is important to the effective delivery of drugs to a local region targeted with ultrasound. As very few regions of the body receive 100% of the cardiac output of the heart, only a fraction of the total number of microcapsules injected into the circulatory system will reach target regions such as the liver, a tumor, etc., on the first circulating pass. In order to effectively dose the target region, the microcapsules need to recirculate with sufficient half-life to eventually reach the target after a number of cardiac passes. The smaller the fraction of total cardiac output received by the target tissues, the greater the need for extended half life of the microcapsule to achieve significant delivery of drug to the target. In the case where the microcapsules are targeted in the tissues with biological agents such as antibodies or by mechanical trapping, half-life of the agent becomes less important as the microcapsules will preferentially accumulate at the target tissues.

Unlike a free gas bubble or a liposome, a microcapsule with significant shell stiffness exhibits an increased resonant frequency dependent on shell properties. It is important that the microcapsules for ultrasound triggered drug delivery demonstrate precise acoustical characteristics, determined by control of shell mechanical properties and thickness as well as narrow size distribution. Adjustment of the strength of the shell may also be modified with chemical agents such as cross linking agents,

plasticizer, or by the internal pressure within the microcapsules.

Microcapsules are preferably constructed as described below. The outer layer of the shell which is exposed to the blood and tissues serves as the biological interface with the body, and so it will be made of a biocompatible material which is amphophilic, that is, it has both hydrophobic and hydrophilic characteristics. The preferred materials are blood compatible polymers or proteins such as polyethylene glycol, polymers and co-polymers, gelatin, human serum albumins or globulins, either derived from humans or having a structure similar to the human protein. The mechanical properties of the outer layer may also be modified, such as by cross linking, to make the microcapsules stable to physiological pressures, to provide a particular resonance frequency for a selected harmonic of the diagnostic imaging system, or to provide stability to a threshold diagnostic imaging level of the ultrasound energy.

The threshold of fragility and drug release is an important consideration in localizing drugs with ultrasound. Microcapsules containing drugs should be resistant to rupture and inadvertent drug release by normal physiological pressures or by ultrasound conditions of the beam passing through tissues not at the target region. By normal physiological pressures, it is meant those pressures encountered in vivo, including pressures within the heart, arteries as well as compressive pressures of passing through constrictions such as capillaries. At minimum, in the use of microcapsules within the circulatory system, the microcapsules should be resistant to normal intracardiac pressures. For example, albumin microcapsules filled with air (Albunex, Molecular Biosystems, Inc.) have been reported to "disappear" in significant amounts in the left ventricle (Gottlieb, et al., 1984), potentially causing problems in use as a drug delivery system to regions other than the left ventricle or with drugs with significant cardiotoxicity.

The inner shell will be a biodegradable polymer, which may be a synthetic polymer. An advantage of the inner shell is that it provides additional drug delivery properties to the microcapsule which are not provided or are insufficiently provided by the outer layer, or enhances mechanical properties not sufficiently provided by the outer layer, without being constrained by surface property requirements. The polymer

may be selected for its modulus of elasticity and elongation, which define the desired mechanical properties. Typical biodegradable polymers include polycaprolactone, polylactic acid, polylactic-polyglycolic acid co-polymers, co-polymers of lactides and lactones, such as epsilon-caprolactone, delta-valerolactone, polyamides, polyhydroxybutryrates, polydioxanones, poly-beta-aminoketones, polyanhydrides, poly-(ortho)esters, polyamino acids, such as polyglutamic and polyaspartic acids or esters of polyglutamic and polyaspartic acids. References on many biodegradable polymers are cited in Langer, et. al. (1983) *Macromol.Chem.Phys.*C23, 61-125.

The inner layer permits the modification of the mechanical properties of the shell of the microcapsule which are not provided by the outer layer alone. The mechanical properties of the inner layer any be adjusted to provide varying threshold levels of microcapsule rupture with ultrasound conditions such as output power. Moreover, the inner layer may provide a drug carrier and/or drug delivery capacity which is not or provided by the outer layer. For use as an ultrasonic contrast agent, the inner layer will typically have a thickness which is no larger than is necessary to meet the minimum mechanical or drug carrying/delivering properties, in order to maximize the interior gas volume of the microcapsule. Generally, the greater the gas volume within the microcapsule the better the echogenic properties.

For use as an ultrasonically triggered drug delivery system, the inner layer can be varied in thickness to provide varying thresholds to rupture, allowing a threshold release characteristic to be utilized. In addition, the mechanical properties of the inner layer such as ultimate elongation, viscous damping, stress at failure and fatigue properties can be tailored by material selection.

The combined thickness of the outer and inner layers of the microcapsule shell will depend in part on the required mechanical and drug carrying/delivering properties, but typically the total shell thickness will be in range of about 25 to 750 nm.

The microcapsules may be prepared by an emulsification process. Due to the amphiphilicity of the material forming the outer layer, stable oil/water emulsions may be prepared having an inner phase to outer phase ratio approaching 3:1 without phase inversion which can be dispersable in water to form stable organic phase droplets

without the need for surfactants, viscosity enhancers or high shear rates.

Two solutions are prepared, the first being an aqueous solution of the biocompatible material to form the outer layer, referred to as the biomaterial. The second is a solution of the polymer which is used to form the inner layer, in a relatively volatile water-immiscible liquid which is a solvent for the polymer, and a relatively non-volatile water-immiscible liquid which is a non-solvent for the polymer. The relatively volatile water-immiscible solvent is typically a C5-C7 ester, such as isopropyl acetate. The relatively non-volatile water-immiscible non-solvent is typically a C8-C20 hydrocarbon such as decane, undecane, and the like. In the second solution containing the polymer for the inner layer, the polymer and water-immiscible solvents are combined so that the polymers fully dissolve and the two solvents are miscible with agitation. The polymer solution (organic phase) is slowly added to the biomaterial solution (aqueous phase) to form a liquid foam. Typically about three parts of the organic polymer solution having a concentration of about 0.5 to 10 percent of the polymer is added to one part of the aqueous biomaterial solution having a concentration of about 1 to 20 percent of the biomaterial. The relative concentrations of the solutions and the ratio of organic phase to aqueous phase utilized in this step essentially determine the size of the final microcapsule and wall thickness. After thorough mixing of the liquid foam, it is dispersed into water and typically warmed to about 30 - 35 °C with mild agitation. While not intending to be bound by a particular theory, it is believed that the biomaterial in the foam disperses into the warm water to provide an emulsion of the polymer in the organic phase encapsulated within a biomaterial envelope. To render the biomaterial envelope water insoluble, a cross linking agent, such as glutaraldehyde, is added to the mixture to react with the biomaterial envelope and render it water insoluble.

Since at this point the inner core contains a solution of a polymer, a solvent and a non-solvent with different volatilities, as the more volatile solvent evaporates the polymer precipitates in the presence of the less volatile non-solvent. This process forms a film of precipitate at the interface with the inner surface of the biomaterial shell, thus forming the inner shell of the microcapsule after the more volatile solvent has evaporated. The core of the microcapsule then contains predominantly the

organic non-solvent. The microcapsules may then be isolated by centrifugation, washed, formulated in a buffer system, if desired, and dried. Typically, drying by lyophilization removes not only the non-solvent liquid core but also the residual water to yield air-filled hollow microcapsules.

If the formulation is to contain a drug-containing core, the microcapsules may be soaked in a solution of the drug so the solution diffuses into the interior. To provide microcapsules having a solid core containing a drug, thicker inner layers may be formed to occupy more or all of the interior volume. Then, by later soaking in the drug-containing solution, the inner solid core will absorb the drug and serve as a solid reservoir. Alternatively, the drug may be dissolved in the microcapsule wall material during the microcapsule forming process, or the drug may be incorporated into the polymer solution during microcapsule fabrication.

The microcapsules may be ruptured by ultrasonic energy to release the trapped drug and gases into the blood stream. During the formulation process the microcapsules may be prepared to contain various gases, including blood soluble or blood insoluble gases.

The materials used for the microcapsule shell, as well as the thickness, may be chosen so as to achieve resonance at a particular frequency, a wave form or the rupturability at a certain combination of energy intensity and duration. Selection of the appropriate mechanical strength of the microcapsule shell allows imaging at conditions which do not trigger drug release, but drug release may be triggered during imaging by altering the ultrasound characteristics. These characteristics are important for controlling and localizing release, especially as matched to the localization of the threshold ultrasound conditions for drug release.

Any of a variety of therapeutics may be encapsulated in the microcapsules. By therapeutic, as used herein, it is meant an agent having a pharmacological or diagnostic effect on the patient. As used herein, the term therapeutic agent is synonymous with the term drug.

The frequency required to resonate a free gas bubble can be calculated according to the following formula:

$$f = \frac{(3kP/\rho)^{1/2}}{\pi d}$$

Wherein f represents the resonant frequency, d represents the diameter of the bubble or microcapsule, k represents the ratio of the specific heat at constant pressure and the specific heat at constant volume of the gas within the microcapsule (the value is a constant of about 1.4 in the case of nitrogen or oxygen), P represents the pressure applied to the liquid surrounding the microcapsule and ρ represents the specific weight of the liquid. The formula is an experimental and theoretical formula in the adiabatic state in which the viscosity of the liquid (typically water) and the surface tension are disregarded.

However, this formula is inappropriate for a microcapsule with a shell with significant mechanical properties and therefore the formula must be modified as follows to include the properties of the shell:

$$f = \frac{((3kP+E')/\rho)^{1/2}}{\pi d}$$

where $E' = 8Eh/d$

Wherein E is the material modulus and h is the shell thickness.

A plot of the equation for a typical microcapsule of 3.5 microns diameter and 0.25 micron shell thickness demonstrates the relationship between the resonant frequency of the microcapsule and stiffness of the shell material.

From the equation, it is apparent that microcapsules typically have an increased resonant frequency for a given size as compared to free gas bubbles, once the mechanical properties of the shell become significant. Also due to the shell, variations in wall thickness and diameter of a microcapsule population produce a distribution of acoustical response, reducing precision in triggering drug release. In order to control and localize drug release, the acoustical characteristics of the microcapsules and the ultrasound field are necessarily precise and complementary.

The resonance frequencies or wave forms required to rupture the

microcapsules according to the present invention may be determined empirically by making a series of formulations with precise acoustic characteristics, using the desired shell materials and the drug, then applying a range of frequencies, wave forms and intensities *in vitro* to determine the appropriate energies, frequencies and wave forms to be utilized on the subject to rupture of the microcapsules *in vivo* at the desired region within the body.

Referring to Figure 2, the reference numeral 2 represents gas-containing microcapsules containing a therapeutic agent. A suspension containing the microcapsules 2 is injected via syringe 3 into a subject 1 intravenously through a hypodermic needle or an intravascular catheter.

An ultrasonic probe 4 is shown for transmitting and receiving a diagnostic ultrasonic wave and the acoustic characteristics and distribution of the microcapsules in the body.

An ultrasonic wave transmitting and receiving circuit 5 comprises a wave form generator or alternatively, a pulser and function generator, or frequency oscillator, for forming an ultrasonic beam which is transmitted from an ultrasonic oscillator in the probe 4 to the object 6 being examined, a transmission/reception delay circuit, the phasing circuit composed of an amplifier amplifying a signal produced by converting the echo received from the object 6 being examined into an electric signal by the ultrasonic oscillator, a wave delay circuit for forming an ultrasonic beam of the received wave by adding the echo signals received by the ultrasonic oscillator after matching the phases, an adder, and the like. An analog/digital converter 7 converts the video echo signal input from the ultrasonic wave transmitting and receiving circuit into a digital signal, a scan converter 8 repeats the operation of writing and reading the output signal of the converter 7 in correspondence with the scanning line of the ultrasonic beam and supplies the output to a picture memory 9. The digital/analog converter 10 generates a video signal from picture memory data through digital/analog conversion and a video monitor 11 provides a display for the video signal to read. A computer 12 calculates the various acoustic parameters from the digital signal output from the analog/digital converter 7.

A resonance ultrasonic wave generator 13 is driven on the basis of the known

resonance frequency of the microcapsule 2 in accordance with a command of the computer 12 or by manual operation. The resonance ultrasonic wave generator 13 includes a wave form generator, a function generator or a frequency oscillator for forming an optimal frequency including the resonance frequency or harmonic ratio of the microcapsule 2 in the object 6 being examined, a transmission/reception delay circuit producing an ultrasonic beam which is transmitted to the object 6 being examined. A color encoder 14 may be utilized on which the display screen of the ultrasonic diagnostic apparatus and the monitor screen for drug release are superimposed. The color displays of the distribution of the gas containing microcapsules, the resonance frequency, the resonance ultrasonic beam, the rate of drug release, the cumulative drug released, the sound field, and the like are superimposed with an ordinary picture, the computer 12 executes and controls a series of the above operations.

Referring to Figure 3, a transducer 15 is designed with multiple focal points 16, 17 for triggering microcapsule drug release within an imaging field 18.

An advantage of the present invention is that by using microcapsules with rupture dependent upon the diameter, shell thickness, and shell mechanical properties, the concentration of the sound energy may be improved so one can control the drug release at an intensity of an ultrasonic wave which does not affect the living subject and minimizes drug release outside of the target region.

The drug is released by either being freed from the inner core by rupture of the microcapsule to expose the inner drug containing layers to the bloodstream or by cavitation effects which allow fluids to be forced into the microcapsule causing dissolution and release of the active agent.

Typically, the microcapsules will be introduced intravascularly by injection, but they also may be injected intra-arterially. The microcapsules may also be injected interstitially or directly into any body cavity.

A useful dosage of the therapeutic agent will be administered and the mode of administration will vary depending upon the age and weight of the subject and upon the particular therapeutic application intended. Typically, the dosage is initiated at a lower level and increased until the desired therapeutic affect is achieved.

In one embodiment, the transducer or multiple transducers may be incorporated into a wearable object to treat a selected region or organ to alleviate the need for manual placement of the transducer and to facilitate concentration of the ultrasound at the target tissues.

As an alternative to activation of the drug delivery microspheres using externally placed ultrasound transducers, the transducer may be incorporated into the distal section of a cannula; or be implanted, within the body, near the target site for the active component delivery. In the first case, an intravascular ultrasound catheter is used to provide the specific ultrasound energy required to disrupt the microcapsules as they pass the catheter being carried by normal blood-flow. The transducer may include long axial transducer sections or combinations of frequency generating elements. The use of such a system provides for the treatment of target sites downstream from the catheter and in places that standard ultrasound imaging would be impaired, in the lungs, for example.

In the case of an implanted transducer, an ultrasound transducer is surgically implanted within the body, at or near the target site for treatment. The transducer may be powered by induction means through the body wall such that it is inert at all times except during use. The active component containing microspheres are injected into the body intravascularly, and the transducer energized to disrupt the microcapsules at the target site. The method is useful for longer term or chronic treatment of a target site.

Although ultrasound energy is preferred for activation of the microcapsules, other forms of energy may be utilized, if necessary, to cause rupture. For example, if a sufficient acoustic window is not available, such as in the lung, microwave radio frequency energy may be utilized. In magnetic induction, an oscillating magnetic field may also be used to create heating of the microcapsules. This may be accomplished with an external magnetic field and magnetic probes implanted within the patient or within the region to be treated.

Preferably the microcapsules possess a reflectivity greater than about -40 dB and preferably between about -10 and about -35 dB. Within these ranges, the highest reflectivity of the microcapsules of the invention is exhibited by larger microcapsules,

by higher concentrations and/or when higher ultrasound frequencies are employed.

Preferably the microcapsules have a peak resonance frequency of between about 1 MHZ and about 40 MHZ, and a resonance bandwidth of 4 MHZ or less. Resonance bandwidths of 2 MHZ or less are preferred. The peak resonant frequency of a gas filled microcapsule will vary depending upon the diameter, material and thickness of the shell and composition of the gas, with the larger microcapsules having a lower resonance frequency than smaller ones, given the same shell materials and thickness. Typical peak resonance frequencies are 2 MHZ or more.

Although application of the various principles will be readily apparent to those skilled in the art, under the guidance of the present disclosure, for a gas-filled microcapsule, typically about 1 to about 8 microns in mean outside diameter, the resonant frequency will generally be in the range of about 1.8 to 40 MHZ. The power can be delivered to a selected focal zone within the target region and gas filled microcapsules may be made to release the therapeutic agent. For larger diameter gas filled microcapsules, typically greater than about 3 microns in mean outside diameter, a lower frequency transducer may be more effective in accomplishing therapeutic release. A lower frequency transducer, for example, 0.5 MHZ to 15 MHZ may be selected to correspond to the resonance frequency or harmonic ratio. Utilizing cavitation to release and/or activate the drug within the microcapsules, lower frequency may be used, as cavitation occurs more effectively at lower frequencies.

In use of diagnostic ultrasound to monitor the location of the gas filled microcapsules, one or several pulses of sound may be used and the machine may be paused between pulses to receive the reflected sonic signals. In the rupturing of microcapsules, a distinct ultrasound pulse is received which can be used to calculate the number of microcapsules releasing drug and the cumulative microcapsules triggered.

It is important in drug therapy to know the amount of active drug delivered in relation to dose-response characteristics. In the use of ultrasound triggered drug release from circulatory microcapsules, the distribution of encapsulated drug may be estimated from the intensity of the backscattered ultrasound signal. However, due to the flow of microcapsules in and out of the target zone and with the pulsed ultrasound

used in imaging, not all of the encapsulated drug may be released. With the acoustic differences in various regions of the body and from patient to patient, calculation of the active drug released is a key aspect of a clinical method to prevent under and over dosing. Measurement of drug release from microcapsules provides significant advantage for practical medical use.

The sound energy may be pulsed but, continuous wave ultrasound energy is preferred for maximal triggering of drug release from the microcapsules. If pulsing is employed, sound will preferably be pulsed in echo trained links of at least about 3 wave periods and preferably be pulsed in echo trained links of at least about 5 wave periods at a time.

Either fixed frequency or modulated frequency sound may be used. For example, a high to low pulse with an initial frequency of 10 MHZ of sonic energy may be swept with increasing power from one to five watts. Focused, frequency modulated, high energy ultrasound may increase the rate of local gas expansion within the microcapsules, rupturing them to provide local delivery of therapeutics.

The frequency of the sound used preferably varies between about 0.01 to about 40 MHZ. Frequency ranges between about 0.01 and 15 MHZ are preferred. Therapeutic frequencies of about 0.02 to 1.2 MHZ may be used. Commonly used diagnostic imaging frequencies are about 1.8 to 7.5 MHZ.

The mode of delivery of the energy to rupture the microcapsules will generally fall into three embodiments of the present invention. The first is to utilize controlled ultrasound energy intensity for rupturing the microcapsules. The frequency of the ultrasound energy input, as applied to all of the described embodiments are typically in the range of about 0.01 MHZ to 40 MHZ. Microcapsules in this case serve as a common ultrasound backscatter platform until the energy reaches a threshold point sufficient to disrupt the microcapsule. Various mechanisms may be employed to render the energy intensity to the point of capsule disruption, for example, by the use two or more emitting transducers at some angle between them. The emission wave form and the transducer foci will be determined such that the additive energy density/wave superposition at the beam convergence is sufficient to cause disruption of the microcapsules. Another configuration may be an annular beam transducer such

that the wave form emission is controlled electronically to produce a spot focus of sufficient energy.

A second embodiment relates to the use of controlled frequencies for interaction with a microcapsule. A specific frequency for example may be used to excite an agent-containing microcapsule to the point of disruption at a resonant frequency. The microcapsule is constructed such that it acts as a signal backscatterer at frequencies commonly used for ultrasound imaging, but undergoes severe oscillation mode shape transitions at the resonant frequency. The microcapsule is rigid enough such that the resonant oscillation causes mechanical failure of the capsule material, thus releasing the therapeutic agent. Alternatively, a microcapsule may be made to have physical characteristics to disrupt under the influence of multiple frequencies or harmonics of a primary frequency. In this instance, a transducer, made to operate at multiple frequencies may be utilized. Thus, a primary frequency may be used for imaging purposes and a secondary frequency may be used at an appropriate frequency to disrupt the microcapsule.

Alternatively, application of sub or super harmonics of a primary frequency may be used to apply the necessary energy for this purpose with microcapsules having the appropriate mechanical properties.

In a third embodiment of the invention, a wave form package within an ultrasound pulse is utilized to achieve high amplitude resonance in order to increase the microcapsule interaction with the ultrasound field for imaging purposes, as well as to disrupt the microcapsule for delivery purposes. Typical ultrasound imaging utilizes wave forms of three or more periods within an ultrasound pulse at the operating frequency. However, microcapsules may be fabricated with increased mechanical properties to require more than seven periods in the pulse in order to reach non linear resonance. Thus, a mode of operation may be utilized to entail the use of low period wave form pulses for imaging and then insonating the microcapsules with higher period wave form pulses to achieve high oscillatory resonance for microcapsule disruption. Multiple transducers or transducer elements within a single transducer may be focused to create a high period wave form pulse spatially focused on the target area. Other variations include the excitation of the microcapsules with a series of

wave form pulses with each succeeding pulse having an increase in wave period. The increase in wave period will be linear, geometric or some other mathematical progression. The upper limit on the number of wave periods within an ultrasound pulse may be set by the intensity and repetition rate of the pulse such as not to exceed the allowable diagnostic dosage for exposure to ultrasound energy to the subject. Alternatively, a wave form pulse in which one or more of the wave periods is of successively higher amplitude than the others may be utilized.

Suitable beam focusing methods include variation of phase delay as described in US Patent 4,938,217, differential frequency response as described in US Patent 5,678,554, variation of time delay as described in US Patent 4,542,653, and electronic element focusing as described in US Patent 5,492,134.

Various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to follow within the scope of the appended claims.

EXAMPLE 1

Controlled Rupture of Microcapsules with Ultrasound

An experiment was performed to determine the ultrasound power threshold required to disrupt the capsule of a bilaminate microcapsule (POINT Biomedical biSphere). The experimental system was set up as follows. A water tank 18" long by 12" wide by 4" deep was constructed using acrylic side walls and a plate glass bottom. A liquid flow loop was constructed to circulate the microsphere agent under test, consisting of a length of vinyl tubing, running through a peristaltic pump and connected to a section of thin walled silicone tubing to create a loop. The imaging was performed with a Hewlett-Packard Sonos 2500 ultrasound scanner. A harmonic imaging transducer (1.8/3.6 MHZ) was connected to the Sonos 2500, and the transducer head mounted on a test stand such that only the transducer face was immersed in the test tank. The flow loop was filled with de-gassed de-ionized water and the transducer was positioned to image the silicone tube.

With the transducer properly aligned, the Sonos 2500 parameters were

adjusted to give a clear image of the flow tube. The peristaltic pump was set to the lowest speed setting. After the set-up was complete, the Sonos 2500 was then switched to harmonic imaging mode (1.8/3.6 MHZ) to observe the harmonic image response of the flow system. Without any contrast agent in the system, the silicone flow loop was barely visible. A small amount of the microcapsule suspension was injected into the flow loop. Under harmonic imaging mode, the contrast agent was immediately visible flowing through the silicone imaging tube.

The Sonos 2500 was then switched into Pulse Wave (PW) Doppler mode and a region of interest for the PW scan was centered on the apex of the silicone flow tube. Under PW mode, the ultrasound scanner converts the Doppler signal into an aural signal within the range of human hearing, and displays a repeating time vs intensity scan for the received signal. With the contrast agent flowing through the imaging tube, the transmission power was varied and the results evaluated on the ultrasound scanner screen and by listening to the aural signal from the converted data. At transmission power below a mechanical index of 1.1 (MI), the PW received signal is a steady baseline, and the aural signal is a steady flow sound overlaid by the repetitive sound of the peristaltic flow from the pump. A sample of the flowing liquid was withdrawn from the injection port and visualized under a standard light microscope. The microcapsules were still intact.

The transmission power was then increased above 1.1MI and the signal scan showed increasing high frequency spikes indicating the disruption of the capsules of the agent under test. The quantity of capsule disruption increased steadily with increasing power, up to the maximum output. The aural signal was a series of "click" sounds which also increased with increasing power. Again, a sample of the flowing liquid was withdrawn from the loop and checked with a light microscope. After ultrasound power input greater than 1.1MI, the majority of the microsphere capsules appeared to be disrupted in some manner, usually fractured along an spherical plane, but in some cases reduced to wall fragments.

The results of the experiment were repeatable, even with the same sample flowing in the loop, i.e. decreasing the power below the 1.1MI threshold stopped the capsule disruption and increasing back above the 1.1MI threshold began disruption, a

process that could be repeated many times until all of the agent capsules under test had been disrupted.

EXAMPLE 2

Effect of Ultrasound Intensity and Wave Period on Microcapsule Resonance

The experiment was designed to determine the backscatter response of an acoustically tailored microcapsule to increasing amplitude and wave period of a sinusoidal waveform. The experiment utilized a Stanford Research Systems (SRS) DS345 30Mhz Function Generator to generate waveforms and to power a Panametrics 2.2Mhz immersion transducer. A second 2.2Mhz transducer was set-up as the pulse receiver and connected to a Tektronix TDS 540B oscilloscope to acquire the backscatter signal and perform FFT's. The microspheres under test were constrained in an acrylic test chamber with a 2 cm path length and 0.0035 inch thick mylar acoustic windows. Waveforms were created using the SRS arbitrary waveform generator software. Ultrasound pulses of sinusoidal waveforms with exponential attack and decay of 2.6Mhz were created, with wave periods (number of sine waves) of 4, 7, 10 and 14 respectively. The ultrasonic pulses were sent from the SRS DS345 at peak to peak output voltages of 1, 2, 5, 7 and 9.5 volts. The resulting backscattered signals were obtained with the oscilloscope and the fourier transforms performed. The results indicate a threshold in both wave periods and voltage that is required to excite the microspheres into non-linear resonance for drug release.

EXAMPLE 3

Fragility Threshold of Gas Filled Microspheres as a Function of Ultrasound Power

An experiment was conducted to determine fragility threshold of gas filled microspheres as a function of ultrasound power. The experiment consisted of a Hewlett-Packard 5500 ultrasound scanner using an S4 transducer in harmonic A mode (send frequency of 1.8 MHZ and received frequency of 3.6 MHZ), imaging an 8mm diameter channel in an ATS Model 524 Doppler Flow Phantom. A constantly stirred reservoir was filled with degassed water and gas filled microspheres in a dilute

solution. A peristaltic pump set up to deliver a constant flow rate was connected between the reservoir and the flow phantom channel. The outflow of the channel was directed to a waste container. The HP 5500 was set up to image the channel and the system controls set-up to be able to control output power from a mechanical index (MI) of 0 to 1.6. The scanner was set-up in pulse wave (PW) mode to graphically display the doppler signal from a region of interest (ROI). When a microsphere passes through the ROI with the power below the fragility threshold of the microsphere, only doppler flow signals are seen on the display. As the power is increased beyond the point where the microspheres are subject to destruction by ultrasound, large amplitude spikes are seen on the PW display from the destruction events of the microspheres. Using the audio output of the system, one can hear the distinctive "clicks" of destruction events.

Four dual walled, nitrogen filled microsphere formulations were tested. The microspheres consist of a biopolymer outer wall and a synthetic polymer inner wall. The outer wall composition was held constant (albumin) while the inner wall material and thickness was varied. Two inner wall polymers were used, polylactide and polycaprolactone. Two different inner wall thickness of each polymer type were tested. The formulations under test were reconstituted from lyophilized cakes and then pipetted into the reservoir. The pump was started and the agent allowed to flow through the channel. The power level of the HP5500 was increased in 0.1 MI increments from around 0.2 MI up to 1.6 MI. The power level where the doppler signal began to indicate microsphere destruction events was recorded. This is an indication of the fragility threshold of the microsphere.

Material	Wall Thickness	Fragility Threshold
Polycaprolactone	54nm	0.5 MI
Polycaprolactone	135nm	0.7MI
Polylactide	108nm	0.7MI
Polylactide	189nm	0.9MI

The results show that polylactide inner walled microspheres require more

power to cause destruction of the capsule than the polycaprolactone microspheres; and that the thicker walled microspheres require more power to destroy than the thinner walled microspheres. The results of the experiment clearly show that the fragility threshold of the microsphere can be controlled by the composition and construction of the capsule. In the case of dual walled microspheres, the fragility of the agent for drug delivery may be tailored by the inner polymer wall properties within the range of diagnostic ultrasound power levels.

WHAT IS CLAIMED IS:

1. A method of delivering pharmacological agents to a region within a subject comprising the steps of:

introducing microcapsules incorporating a pharmaceutical agent into the bloodstream of said subject, said microcapsules comprising a shell of at least one layer containing a polymer for retaining said pharmacological agent and which are rupturable at a pre-determined threshold ultrasound condition of power, frequency and wave form but not ruptured at normal physiological pressures;

(a) monitoring the location of said microcapsules within said subject to determine the presence of said microcapsules at said region;

(b) directing at said region, ultrasonic energy at a frequency, intensity and/or wave form sufficient to induce the release of said pharmacological agent in said region from said microcapsules to achieve a pharmacological effect.

2. A method according to claim 1 where in said threshold ultrasound condition is a power, frequency and wave form sufficient to provide a mechanical index of about 0.2.

3. A method according to claim 1 wherein said threshold ultrasound condition is a power, frequency and wave form sufficient to provide a mechanical index of about 0.5.

4. A method according to claim 1 wherein said threshold ultrasound condition is a power, frequency and wave form sufficient to provide a mechanical index of about 1.0.

5. A method according to claim 1 wherein said threshold ultrasound condition is a power, frequency and wave form sufficient to provide a mechanical index of about 1.5.

6. A method according to claim 1 wherein said microcapsules comprises a bi-layer shell.

7. A method according to Claim 1 wherein said ultrasonic energy is produced by a plurality of transducers focused at said region whereby the intensity and wave superimposition at the point of convergence of the emitted ultrasonic beams is sufficient to rupture said microcapsules.

8. A method according to Claim 1 wherein said ultrasonic energy is produced by electronically focusing of a transducer array to produce a region of ultrasound irradiation above the threshold ultrasound condition to trigger drug release within the image field of said array.

9. A method according to claim 8 wherein said threshold ultrasound condition is a power, frequency and wave form sufficient to provide a mechanical index of about 0.2.

10. A method according to claim 8 wherein said threshold ultrasound condition is a power, frequency and wave form sufficient to provide a mechanical index of about 0.5.

11. A method according to claim 8 wherein said threshold ultrasound condition is a power, frequency and wave form sufficient to provide a mechanical index of about 1.0.

12. A method according to claim 8 wherein said threshold ultrasound condition is a power, frequency and wave form sufficient to provide a mechanical index of about 1.5.

13. A method according to Claim 1 further comprising ultrasonically monitoring the release of said pharmaceutical agent from the microcapsules to

determine rate of release and cumulative dosage released by monitoring microcapsule rupture.

14. A method according to Claim 1 wherein said ultrasonic energy is produced by an annular beam transducer focused upon said region to provide energy within the focal area which is threshold ultrasound above the condition.

15. A method according to Claim 1 wherein said ultrasonic energy is produced by a transducer embodied within the distal portion of a cannula to disrupt said microcapsules as they flow to said region.

16. A method according to Claim 1 wherein said ultrasonic energy is produced by a transducer implanted within the body near said region.

17. A method according to Claim 1 wherein said ultrasonic energy is produced by a transducer affixed to an external, wearable object affixed near said region.

18. A method according to Claim 1 wherein said ultrasonic energy is of a frequency to sufficiently excite said pharmacological agent such that resonance of said agent ruptures said microcapsules.

19. A method according to Claim 1 wherein said microcapsules resonate at harmonic ratios of a primary frequency and said ultrasonic energy is provided by transducers which emit said harmonics sufficient to cause rupture of said microcapsules.

20. A method according to Claim 1 wherein said microcapsules structurally resonate under irradiation of multiple frequencies and said ultrasonic energy is provided by transducers which emit said frequencies to cause rupture of said microcapsules.

21. A method according to Claim 1 wherein said ultrasonic energy is provided by a wave form sufficient to induce resonance sufficiently high to rupture said microcapsules.

22. A method according to claim 21 wherein said wave form is a constant pulse with constant amplitude.

23. A method according to claim 21 wherein said wave form is provided by pulse inversion.

24. A method according to claim 21 wherein said wave form is provided by extending pulse length beyond the number of wave periods used for diagnostic imaging.

25. A method according to Claim 21 wherein said ultrasonic wave form is induced in a spatial target region from multiple transducers or transducer elements of a single transducer.

26. A method according to Claim 21 wherein said wave form required to disrupt the microcapsule consists of 3 or more wave periods.

27. A method according to Claim 21 wherein said wave form required to disrupt the microcapsule consists of 5 or more wave periods.

28. A method according to Claim 1 wherein said microcapsules have a resonant frequency greater than or equal to 2 MHZ.

29. A method according to Claim 1 wherein said microcapsules have a resonant frequency bandwidth of 4 MHZ or less.

30. A method according to Claim 1 wherein said microcapsules have a

resonant frequency bandwidth of 2 MHZ or less.

5

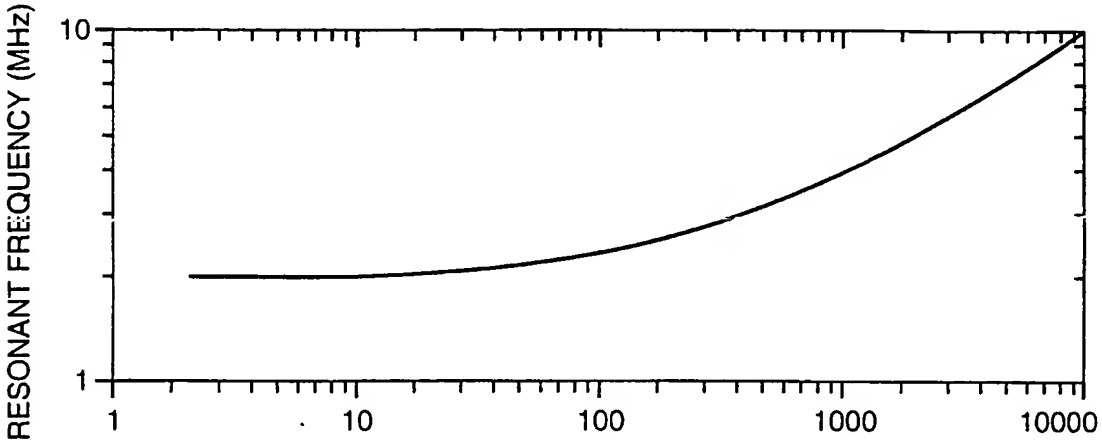
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FIG._1

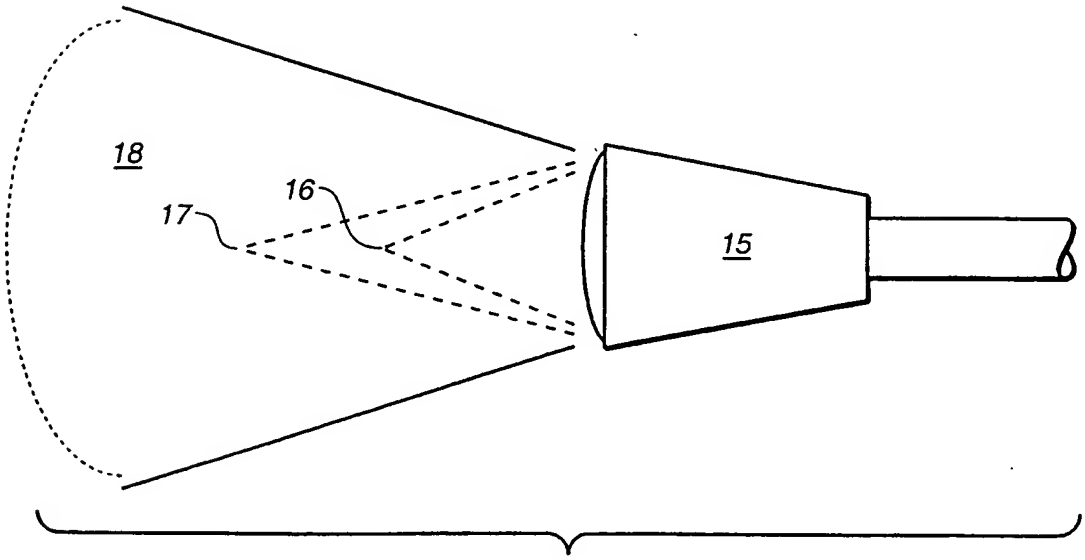
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FIG._3

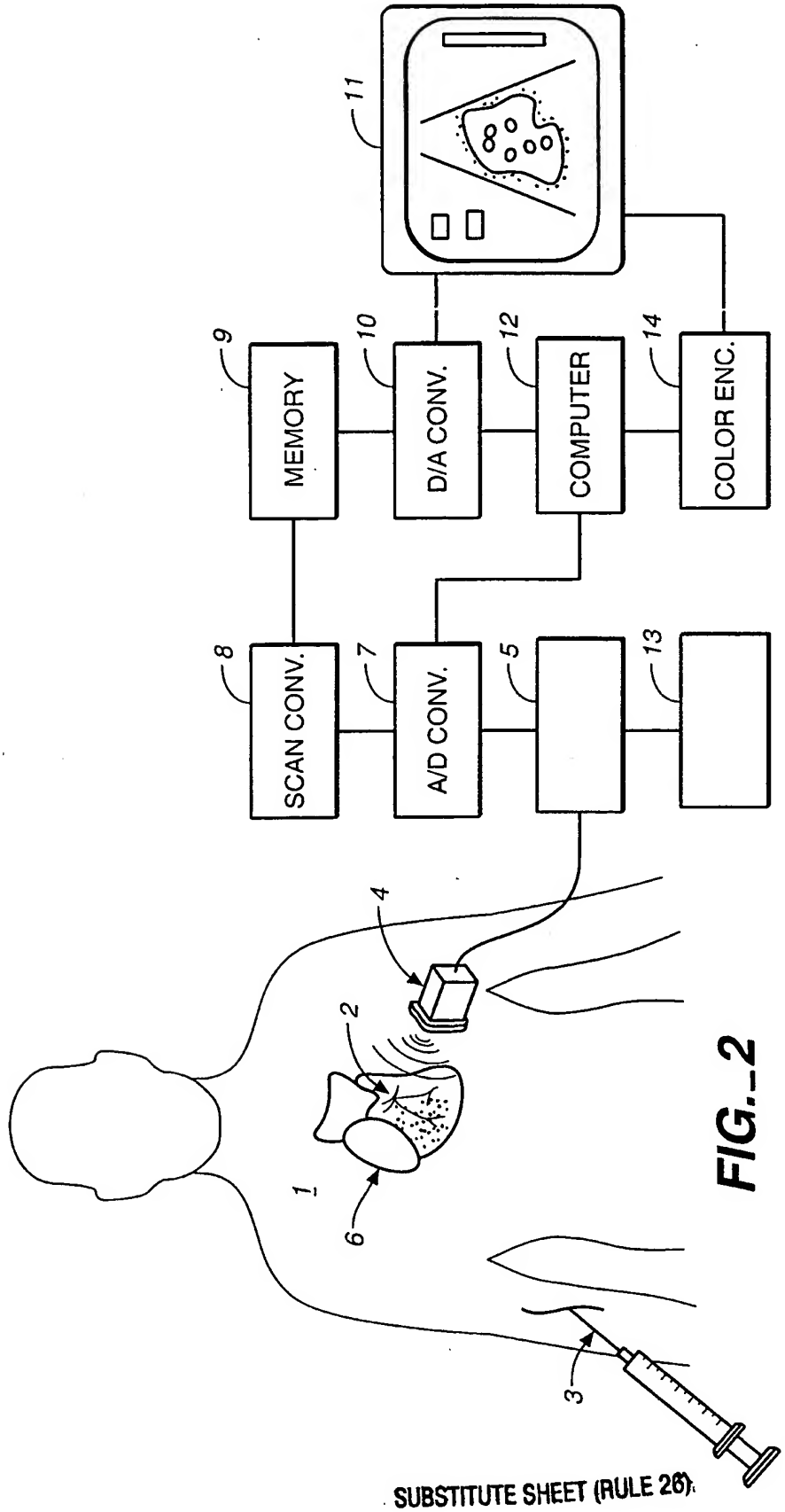
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FIG._2

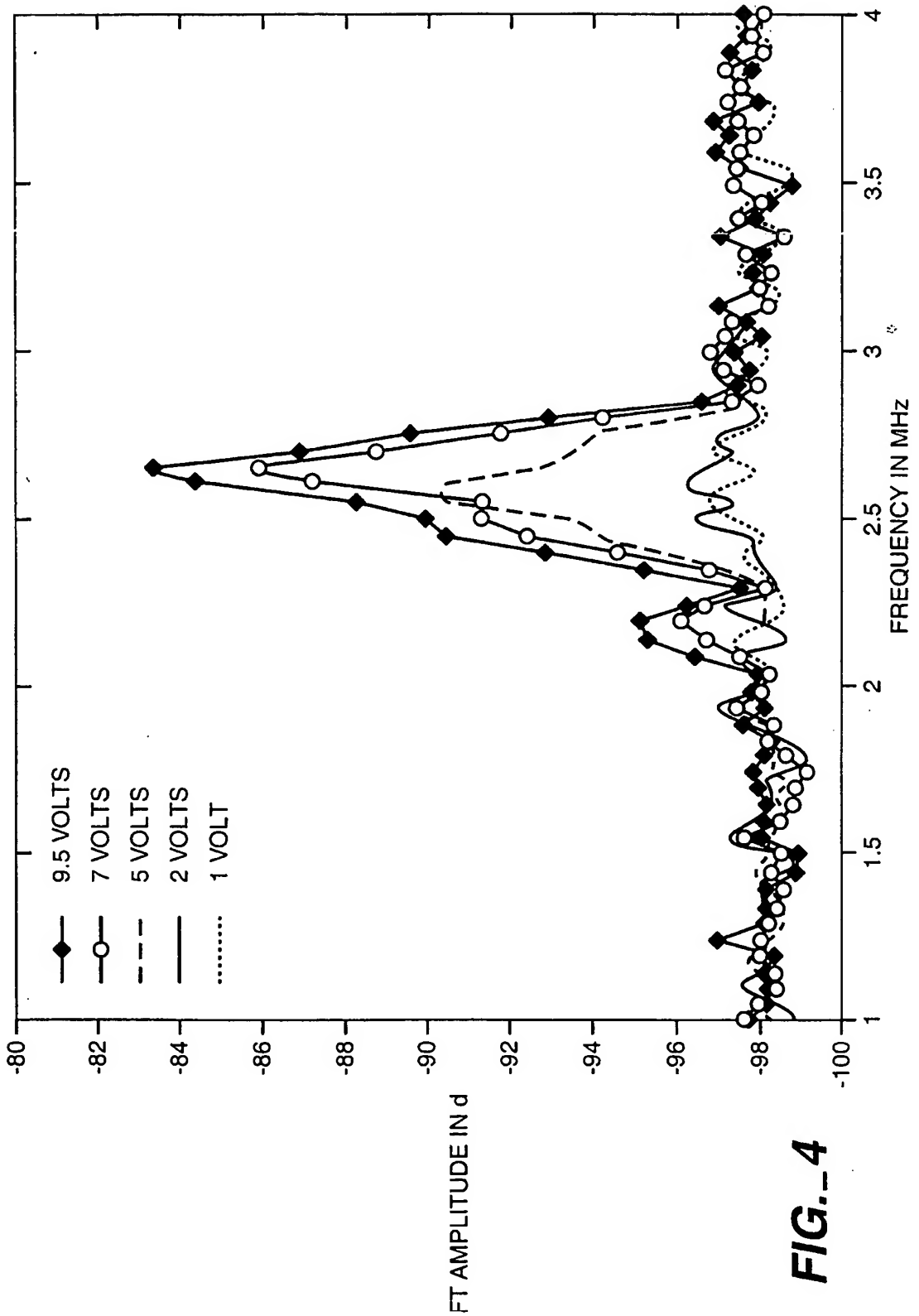
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FIG._4

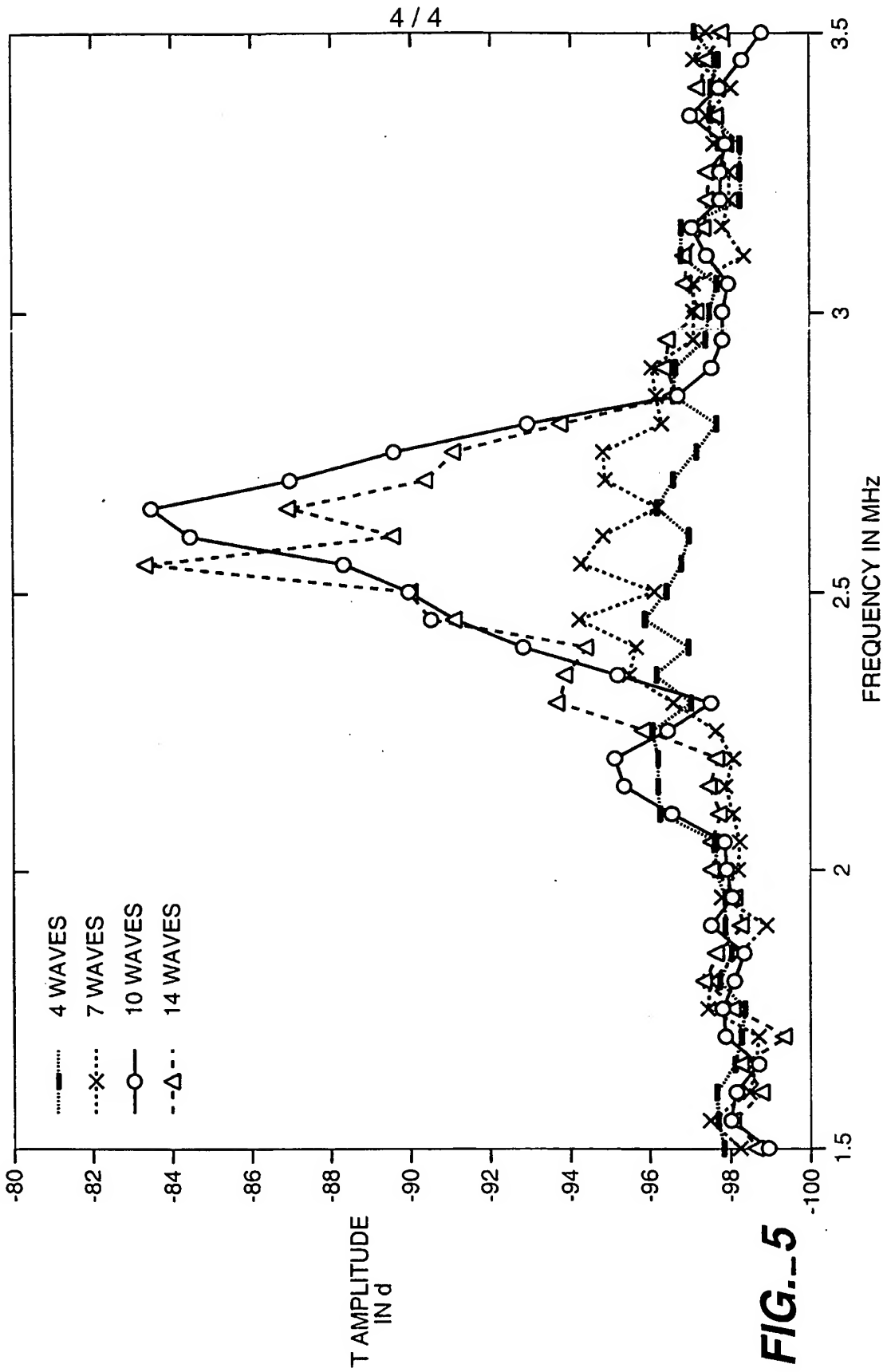
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FIG._5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/02579

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 9/127; A61M 31/00
US CL :424/450; 604/20, 500

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/9.5, 9.51, 9.52; 450, 471, 472, 490; 600/467; 601/3; 604/19, 28, 31, 891.1; 607/99, 112

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,580,575 A (UNGER et al) 03 December 1996, entire document.	1, 6, 7, 13, 16, 19-21, 25, 28-30 --- 2-5, 8-12, 14, 15, 17, 18, 22-24, 26, 27
X --- Y	US 5,190,766 A (ISHIHARA) 02 March 1993, entire document.	1, 13 --- 2-12, 14-30
Y	US 4,441,486 A (POUNDS) 20 April 1984, Abstract, col. 2 lines 1-57, and col. 8 lines 7-29.	7, 8, 19, 20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 22 MARCH 1999	Date of mailing of the international search report 12 APR 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SHARON FINKEL Telephone No. (703) 305-0154

Form PCT/ISA/210 (second sheet)(July 1992)*